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PATENT APPLICATION

MICROFLUIDIC-BASED ELECTROSPRAY SOURCE FOR ANALYTICAL DEVICES

Inventor(s):

Ian David Manger, a citizen of United Kingdom, residing at
516 Hawthorne Avenue, Palo Alto, California 94301

Cunsheng Casey Hao, a citizen of Canada, residing at
10200 Miller Avenue, Cupertino, California 95014

Marc Alexander Unger, a citizen of United States, residing at
2555 Adams Court, South San Francisco, CA 94080

Assignee:

Mycometrix Corporation
213 E. Grand Avenue
South San Francisco, CA 94080

Entity: Small business concern

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
Tel: 415-576-0200

Microfluidic-Based Electrospray Source for Analytical Devices

FIELD OF THE INVENTION

This invention relates to microfluidic devices and methods for using the same. In particular, microfluidic devices of the present invention comprise a fluid flow channel and a means for providing a fluid sample from the fluid flow channel to an analytical device.

BACKGROUND OF THE INVENTION

Recently, microfluidic devices capable of conducting chemical reactions and assays on a single microchip have been developed. However, the method of detection has been mostly limited to laser-induced fluorescence (LIF) because of its simplicity and sensitivity. One of the limitations of LIF is that it requires the analyte of interest to be fluorescent. Since most compounds are not natural fluorophores, LIF is not an ideal detection method. Thus, in order to use LIF detection method, a derivatization step is often required to make compounds of interest amenable to LIF detection.

Mass spectrometry (MS) is currently being investigated as an alternative detection method for microfluidic devices. In this regard, electrospray ionization mass spectrometry (ESI-MS) is particularly suited due to the similarity in flow rates generated by the microchip (i.e., microfluidic device) with those required for ESI-MS. ESI-MS is a powerful tool that has been broadly applied to the structural analysis of biological molecules. In particular, it provides a facile means to interface liquid chromatographic (LC) systems and mass spectrometry (MS), creating a system that integrates separation with structural analysis and molecular identification. The development of LC-MS has revolutionized analytical chemistry and biochemistry.

In the post-genomic era, attention has turned from DNA sequencing to the more complex problem of analyzing how this genetic information directs cell function. The analysis of protein structure and function is one of the keys to this question. In particular, analysis methods currently under development are typically focused on identifying unknown proteins whose presence can be correlated with a function, disease state or reaction to potential drug candidates.

Mass spectrometry is a highly sensitive tool for the analysis of proteins. It enables the masses of fragment ions of proteins or peptides to be determined with high accuracy and with high sensitivity. High mass accuracy enables an accurate and specific sequencing of peptides. In combination with progress in genomic sequencing and bioinformatics, this enables the identification and characterization of unknown components of cells. In tandem with multidimensional gel electrophoresis methods, it provides a means to identify the complement of the proteins expressed by a cell under a defined set of conditions. This totality of expressed proteins is defined as the proteome.

Mass spectrometry is also developing from this simple "mining tool" for providing protein sequence information into more deeply integrated areas, such as functional characterization of biologically important genes, functional proteomics, quantitative mapping of cellular proteins and deciphering protein interaction networks. In addition to sequencing, mass spectrometry is currently the only tool available that can readily detect post-translational modifications (changes to protein structure after synthesis), such as phosphorylation and dephosphorylation and the actions of proteases that each plays critical roles in the control of cellular activity.

Another important MS application is the identification of molecules participating in the formation of macromolecular complexes. The study of molecular interactions is a rapidly developing field. The analysis of protein expression in cells (also known as proteomics) is therefore important in target identification and validation, and in ADME/PK (absorption-distribution-metabolism-excretion/pharmacokinetic) studies. However, such proteomic studies, in which proteins are identified by analysis of enzymatically produced peptide fragments, are expensive and labor-intensive. Technical difficulties exist in both sample separation and sample delivery systems for using ESI-MS in analysis of proteins, primarily because the samples that can be isolated from traditional gel-based electrophoresis are in very limited amounts. This makes them difficult to analyze in a traditional ESI-MS configuration.

To overcome some of the problems created by small sample sizes, interfaces capable of delivering low nanoliter per minute volumes of sample (so-called 'nanospray') to MS have been developed. These extend the time over which a very small amount of sample (e.g., 1 μL or less) can be delivered to the mass spectrometer, providing improved signal/noise ratios and thus sensitivity. However, Nano-ESI-MS is labor-intensive and slow (in current designs, sample loading and set-up of the

electrospray capillary are both manual processes). In addition, it cannot be readily adapted to on-line capillary separation methods such as liquid chromatography or capillary electrophoresis. For these reasons, nanospray is most often used as a "static" or off-line method in which samples are analyzed one-at-a-time, representing a serious bottleneck in applications that requires high throughput. Software that integrates the variety of analytical methods required to perform high throughput analysis using these systems is already available, thus design of a robust multi-use interface is the bottleneck in adapting nanospray to high throughput applications.

Microfluidic device based electrospray sources for use in mass spectrometry have recently been developed; *see for example*, Oleschuk and Harrison, *Trends in Anal. Chem.*, **2000**, *19*, 379-388, and Licklider et al., *Anal. Chem.*, **2000**, *72*, 367-375. However, these methods utilize non-elastic microfluidic devices and require fabricating an electrospray nozzle directly on the microfluidic device or attaching a capillary electrospray emitter to the microfluidic device. Unfortunately, fabrication of an electrospray nozzle directly on the microfluidic device increases the manufacturing complexity, the production time and the cost. Methods for attaching a capillary electrospray emitter to current microfluidic devices also have severe limitations. For example, the junction between the microfluidic device and the electrospray nozzle emitter requires a tight seal to avoid fluid sample leakage. More significantly, it is difficult to attach an electrospray emitter to non-elastic microfluidic device without introducing a certain amount of void volume. Furthermore, the electrospray emitter must be carefully attached to the microfluidic device making mass production using batch processes difficult.

Moreover, in these microfluidic devices the flow of fluid is typically electroosmotically driven or by applying pressure directly on the inlet portion of the microfluidic devices. These fluid flow methods further limit the utility of these microchips. For example, use of electroosmotic flow is incompatibility with many buffer systems, may cause molecular dissociation, and molecules can be damaged or degraded due to exposure to electric fields. Most importantly the ionic buffers required to drive electroosmotic flow interfere with electrospray ionization and limit its usefulness. The use of electric fields is also incompatible applications that demand the use of non-aqueous solvents.

Therefore, there is a need for a microfluidic device which comprises a means for providing a sample of fluid to an analytical device which does not require

fabrication of sample providing means directly on the microfluidic device??. There is also a need for a microfluidic device in which a readily available electrospray emitter can be easily attached. There is also a need for a microfluidic device which does not require electroosmotic flow or electrophoresis or a direct application of pressure on the inlet portion of the microfluidic device.

SUMMARY OF THE INVENTION

The present invention provides a microfluidic device comprising a means for providing a fluid sample from directly from the microfluidic device to an analytical device, and methods for using the same.

In one aspect, the present invention provides a microfluidic device comprising:

(a) a first elastic layer;

(b) a fluid flow channel within said elastic layer; and

(c) a means for providing a sample of fluid from said fluid flow channel to an analytical device.

In another aspect of the present invention, an analytical apparatus for analyzing a fluid sample is provided. The analytical apparatus comprises an analytical device for analyzing the fluid sample and the microfluidic device described above.

Yet another aspect of the present invention is a method for producing a microfluidic device comprising a means for introducing a fluid sample into an analytical device. The method generally comprises the steps of:

(a) producing a first elastic layer of the microfluidic device, wherein the first elastic layer comprises a fluid flow channel; and

(b) integrating a proximal end of a capillary within said fluid flow channel,

wherein a distal end of said capillary comprises said sample introducing means.

Still another aspect of the present invention provides a method for analyzing a fluid sample using an analytical device comprising the steps of:

(a) introducing the fluid sample into the analytical device through a fluid flow channel of a microfluidic device, wherein the fluid flow channel is located within a first elastic layer of the microfluidic device; and

(b) analyzing said fluid sample using the analytical device.

Preferably, the analytical device is a mass spectrometer.

Preferably, the microfluidic device of the present invention further comprises a second elastic layer which is positioned on top of the first elastic layer. In one embodiment of the present invention, the second elastic layer comprises a pressure channel which can act as pumps and valves for controlling the flow of fluid within the fluid flow channel in the first elastic layer. Thus, microfluidic devices of the present invention have significant advantages in both the sample preparation and sample delivery (in scales of nL/min). For example, in sample preparation, the combination of miniaturized valves and pumps on top of the first elastic layer allows one to conduct complex sample preparation processes, thereby circumventing shortcomings (some of which are described above) of electroosmotically driven microfluidic devices.

Other benefits of microfluidic devices of the present invention include reduced manufacturing and operating costs, reduced resource consumption, reduced waste production, and increased throughput (e.g., both by speeding up sequential, individual runs and also by implementing parallel processing). Further advantages of microfluidic devices of the present invention include adaptation of traditional LC packing materials that enable separations to be permitted on the device. The revolutionized sample processing and biochemical analysis provided by the present invention create "flow" or on-line systems, which can be adapted to high throughput methods.

Thus, in one particular embodiment of the present invention, an integrated system of microfluidic device and ESI-MS (i.e., chip-ESI-MS) is used to process and then deliver nanoliter or picoliter scale samples with a uniform low sample flow rate (e.g., nL/min) for direct analysis of the fluid sample which has been prepared using the microfluidic device.

DEFINITIONS

The term "elastic layer" and "elastomeric later" are used interchangeably herein and refer to a material which can be deformed by applying pressure. Preferably, the Young's modulus of the elastic layer is from about 1 Pa to about 1 TPa, preferably from about 10 Pa to about 100 GPa, more preferably from about 20 Pa to about 1 GPa, still more preferably from about 50 Pa to about 10 MPa, and most preferably between about 100 Pa to about 1 MPa. However, elastomeric materials having a Young's modulus outside of these ranges can also be utilized depending on the needs of a particular application.

Unless otherwise stated, the term "liquid chromatography device" includes low pressure liquid chromatography devices (LPLC), medium pressure liquid chromatography devices (MPLC), and high pressure liquid chromatography devices (HPLC).

Unless otherwise stated, the term "electrospray mass spectrometer" refers to electrospray ionization mass spectrometers, including nanoelectrospray mass spectrometers.

The term "electrospray" refers to a method of generating a very fine liquid aerosol (i.e., mist) through electrostatic charging. Such methods are well known to one of ordinary skill in the art. Briefly, a plume of liquid droplets is generated by electrically charging a volume of liquid to a high voltage. The liquid becomes unstable as it is forced to hold more and more charge. When the liquid reaches a critical point (i.e., at critical charge/volume ratio), at which it can hold no more electrical charge, it rapidly dissociates (i.e., blows apart) into a cloud of tiny, highly charged "daughter" droplets. These tiny daughter droplets then fly towards detector which typically has opposite charge or ground potential. As droplets fly about, solvent molecules evaporate from their surface and the daughter droplets can further dissociate due to increased charge/volume ratio.

The term "nanoelectrospray mass spectrometer" refers to mass spectrometers having a low sample fluid flow rate. Nanoelectrospray mass spectrometers have sample fluid flow rate in the range of from about 1 nL/min to about 150 nL/min, and preferably from about 20 nL/min to about 50 nL/min.

The term "directly" as used in reference delivering or introducing a fluid sample from a microfluidic device to an analytical sample refers to a method for introducing a fluid sample to an analytical device without any intervening manual manipulation of the fluid sample. In particular the fluid sample leaving the microfluidic device enters the injection port of the analytical device directly.

The term "circular cross-section" refers to the cross-section of a channel that is a circle, oval, ellipse, or other similarly circular shape.

The term "capillary nozzle" refers to a device which has a capillary tube or similar opening which is used to provide a fluid sample from the microfluidic device to the analytical device. The tip of a capillary nozzle can be tapered or non-tapered. Preferably, the inner diameter of the capillary nozzle is from about 1 μm to about 100 μm,

more preferably from about 10 μm to about 50 μm, and most preferably from about 10 μm to about 20 μm.

The term "integrated" refers to combining a microfluidic device with a means for fluid sample delivery such that the fluid sample is introduced directly to the sample injection site of the analytical device from the microfluidic device.

The term "analyte" refers to a particular compound which is to be analyzed by the analytical device.

The terms "injected" and "introduced" are used interchangeably herein and refer to providing the fluid sample into the analytical device for analysis.

The term "rotary" refers to a configuration in the fluid flow channel which allows circulation of a fluid within a confined region or section of the fluid flow channel.

The term "channel" refers to an empty space within the elastomeric layer in which a fluid can be introduced. Preferably, a liquid is introduced in a fluid channel and a gas is introduced in a pressure channel.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A is a schematic illustration of a microfluidic device comprising a means for delivering a fluid sample directly to an injection port of an analytical device;

Figure 1B is a schematic illustration of an analytical apparatus comprising a microfluidic device having an electrospray capillary interconnected to a mass spectrometer;

Figure 2A is a front view of a microfluidic device comprising a fused silica capillary nozzle which can be used as an electrospray source;

Figure 2B is a top view illustrating a portion of microfluidic device which comprises pumps (dotted line) on a layer above the fluid flow channel (solid line);

Figure 2C is a close-up view of one particular embodiment of an interface between a fluid flow channel in microfluidic device and a capillary tube which is used as a means to deliver a fluid sample to an analytical device (not shown);

Figures 3A and 3B are a schematic illustration of creating an arcuate (e.g., rounded) fluid flow channel elastomer using a photoresist mold;

Figure 4A is a schematic illustration of an elastomeric peristaltic pump located above a fluid flow channel;

Figure 4B is a graph showing pump rate (i.e., fluid flow rate) versus frequency of peristaltic pump of Figure 4A;

Figure 5 is a schematic illustration of a fluid flow channel comprising a sample preparation chamber and reagent injection sites;

Figure 6A is a front view of the first elastic layer integrated with a capillary nozzle;

Figure 6B is a side cross-sectional view of the first elastic layer fitted with a capillary nozzle with dead volume in between the fluid channel and the capillary nozzle;

Figure 6C is a side cross-sectional view of the first elastic layer fitted with a capillary nozzle having a tapered fitting end which reduces the amount of dead volume;

Figure 7A is a perspective view of the first elastic layer having a rectangular cross-section fluid flow channel;

Figure 7B is a cut-away view along 1-1' of Figure 7A showing a tapered portion of fluid flow channel which is designed to reduce the amount of dead volume between the capillary nozzle and the fluid flow channel;

Figure 7C is a front view of the first elastic layer fitted with a capillary nozzle illustrating a possible gap formation between the capillary nozzle and the fluid flow channel;

Figure 8 is another embodiment of attaching a capillary nozzle to a microfluidic device of the present invention through the Z-axis;

Figure 9A is a schematic illustration of one embodiment of using a capillary nozzle as an electrospray;

Figure 9B is a close-up view of the capillary nozzle of Figure 9A;

Figure 9C is a schematic illustration of another embodiment of using a capillary nozzle as an electrospray; and

Figure 9D is a close-up view of the capillary nozzle of Figure 9C.

DETAILED DESCRIPTION

The present invention will be described with regard to the accompanying drawings which assist in illustrating various features of the invention. In this regard, the present invention generally relates to a microfluidic device comprising a means for providing a fluid sample from to an analytical device, an analytical apparatus comprising the same, and methods for using the same. The same numbers in different drawings represent identical elements. The drawings are provided for the purpose of illustrating the practice of the present invention and do not constitute limitations on the scope thereof.

The analytical device **120** can be any device which is used for analyzing a chemical compound. Typically the analytical device **120** has a sample injection port **114** for introducing a sample to be analyzed. While the sample can be in a pure form (i.e., neat), microfluidic devices of the present invention are particularly useful for analyzing compounds which are in a solution.

Preferably, the analytical device **120** is a selected from the group consisting of UV spectrometers, fluorescence spectrometers, IR spectrometers, gas chromatographic devices, liquid chromatographic devices, NMR devices, mass spectrometers and combinations thereof. More preferably, the analytical device **120** is a mass spectrometer. And most preferably, the analytical device **120** is an electrospray mass spectrometer.

The fluid sample providing means **124** can include any device that allows delivery of the fluid sample from the microfluidic device **100** to the analytical device **120**. Exemplary fluid sample delivery devices include the outlet ports of fluid flow channel on microfluidic devices; capillary nozzles (such as electrospray nozzles); needles, and Preferably, the fluid sample device is a capillary nozzle, and more preferably an electrospray nozzle.

Microfluidic devices of the present invention are capable of delivering a very minute amounts of samples to the analytical device, thereby increasing the sensitivity of the analytical device **120**. In particular, microfluidic devices of the present invention are capable of providing a fluid sample to the analytical device at a flow rate of from about 1 nL/min to about 200 nL/min, preferably from about 10 nL/min to about 50 nL/min, and more preferably from about 10 nL/min to about 20 nL/min.

While the present invention is generally described in reference to microfluidic devices for preparing and/or providing fluid samples to electrospray mass spectrometers, it should be appreciated that the present invention is not limited to such. For example, microfluidic devices of the present invention can be integrated with a LC-mass spectrometer, GC-mass spectrometer, liquid chromatography devices, gas chromatography devices, IR spectrometer, UV spectrometer, fluorescence spectrometer, or the like by using a capillary nozzle, needle, or some other fluid sample delivery means which provide a non-aerosol (i.e., mist) liquid samples to analytical devices.

One aspect of the present invention provides a microfluidic device **100** comprising a first elastic layer **25**, a fluid flow channel **18** within the first elastic layer **25**, and a means for providing a sample of fluid **124**, preferably directly, from the fluid flow

channel to an analytical device **120**. Methods for producing microfluidic devices comprising an elastic layer is generally described in U.S. Patent Application Serial No. 09/605,520, filed on June 27, 2000. The first elastic layer **25** of microfluidic devices of the present invention preferably comprises at least two elastic portions, a top portion **20** and a bottom portion **10**. In this embodiment, the fluid flow channel **18** is formed at the interface of the top and bottom portions of the first elastic layer. This is particularly advantageous when forming a fluid flow channel having a circular cross-section for integrating a fluid sample providing means which comprises a capillary nozzle or other devices having a circular or rounded cross-section.

Preferably the inner diameter of the capillary nozzle **14** is from about 1 μm to about 100 μm, more preferably from about 10 μm to about 50 μm, and most preferably from about 10 μm to about 20 μm. The outer diameter of the capillary nozzle **14** is dependent on the width (e.g., diameter) of the fluid flow channel **18** or the portion of fluid flow channel which is integrated with the capillary nozzle.

It is preferred that the inner diameter of the capillary nozzle **14** be substantially similar to the width of fluid flow channel **18**, as this diameter to width matching allows minimal fluid flow disruption and/or pressure differential between the fluid flow channel **18** and the capillary nozzle **14**. Typically, the outer diameter of the capillary nozzle **14** is larger than the width of fluid flow channel **18**; therefore, in order to provide a substantially similar width, the portion of fluid flow channel **18** which integrates the capillary nozzle **14** is constructed such that its width is substantially similar to the outer diameter of the capillary nozzle **14**. It is preferred, however, that the volume of the portion of fluid flow channel **18** that integrates the capillary nozzle **14** be slightly smaller than the volume occupied by the outer dimension of the portion of capillary nozzle, as this arrangement provides a "snug" fit or a hermetic seal. This is particularly useful in microfluidic devices of the present invention as they have an elastic layer which can expand to accommodate the capillary nozzle **14**. It should be appreciated that the amount of expansion possible by the first elastic layer depends on the particular nature of the material used. Alternatively, an adhesive can be used to secure the capillary **14** within the flow channel **18**.

It has been found by the present inventors that to achieve this minute fluid sample flow rate, a microfluidic device that is constructed using multi-layer soft lithography having one or more elastomeric pumps **34** is particularly useful. See for

example, Unger et al, *Science*, **2000**, *288*, 113-116, and U.S. Patent Application Serial No. 09/09/605,520, filed June 27, 2000, all of which are incorporated herein by reference in their entirety. Thus, preferred microfluidic devices of the present invention can further comprise a second elastic layer **30** which comprises one or more pump and/or valve systems. These pumps and valves allow control of fluid flow within the fluid flow channel **18** by selectively closing and opening particular sections of fluid flow channel **18**. Furthermore, the rate of fluid flow within the fluid flow channels can be controlled by these pumps. These pumps are capable of delivering pico- to nanoliter per minute of fluid flow rates though fluid flow channels within microfluidic devices to the capillary nozzle **14**. For example, using the fluid channel arrangement shown in Figure 4A, where the channels are 100 μm wide and 10 μm high, with 30 μm gap between the fluid channel **18** and the pressure channels **50**, the rate of fluid flow through the fluid channel **18** is measured as a function of peristaltic pump **34** (i.e., opening and closing of each pressure channels **50**) frequency. Peristalsis is actuated by the pattern 101, 100, 110, 010, 011, 001, where 0 an 1 indicate "valve open" and "valve closed," respectively. Figure 4B shows the pump rate (nL/s) per peristalsis frequency. By reducing the frequency of the pump **34** and/or the dimensions of the fluid channel **18**, one can easily adjust the fluid flow rate within the fluid channel **18** to pico- to nanoliter per minute.

Preferably, microfluidic devices of the present invention are capable of delivering from about 0.5 nL/min to about 200 nL/min of fluid sample to the analytical device, more preferably from about 10 nL/min to about 50 nL/min, and most preferably from about 10 nL/min to about 20 nL/min. These minute delivery rate allows a very minute quantity of analyte in the fluid sample to be injected into the analytical device **120** over a much longer period of time than otherwise possible using conventional means. This constant stream of the fluid sample over a relatively long period of time allows accurate analysis of minute quantities of analytes.

Another advantage of pump-driven elastomeric microfluidic devices of the present invention over current microfluidic devices that use electrokinetic flow means is that electric fields are not required to drive the flow of the solvent, therefore the rate of fluid flow is composition independent. Moreover, electrokinetic flow requires a high salt concentration buffer solution to affect fluid sample flow. The inclusion of high salt concentrations in the buffer creates problems with ionization in ESI and causes a significant background noise. Since microfluidic devices of the present invention do not

require electric fields, they eliminate the need for a high salt concentration buffer solution, thereby reducing the background noise and increasing the sensitivity of the analytical device. Furthermore, the lack of requirement for a high salt concentration buffer solution also allows the use of non-aqueous solvents, thereby greatly extending the field of application of these devices.

Microfluidic devices of the present invention can also comprise a sample preparation chamber within the fluid flow channel **18**. In this manner, the analyte sample can be prepared and injected into the analytical device **120** directly. The sample preparation chamber can be any configuration which allows preparation of analyte to be analyzed. For example, the sample preparation chamber can be an array of fluid flow channels and pressure channels (i.e., pumps and valves) which can be used in combinatorial synthesis. Exemplary microfluidic devices for combinatorial synthesis are disclosed in Patent Application entitled "Combinatorial Synthesis System," filed on October 3, 2000, by R. Michael van Dam, Marc Unger and Stephen Quake, and further identified as Attorney Docket No. 020174-001600US. As shown in Figure 5, the sample preparation chamber can include or be a rotary fluid flow channel **50** and a means for circulating a fluid (e.g., by using circulation pumps **34'** and closing valves **42A** and **42B**) within the rotary fluid flow channel **50**. The rotary fluid channel **50** can be used to conduct a chemical reaction, an assay, protein degradation, separation, or other sample preparations processes.

For example, chemical reaction can be conducted by introducing reagents through fluid flow channels **18** using one or more sample or reagent injection ports **46A** and **46B** (see Figure 5). The reagents are then pumped by the pump **34** (e.g., see Figure 2B) which can be located anywhere along the fluid flow channel **18**. These reagents can be admixed and allowed to react for a period of desired time by "holding" the reaction mixture within the rotary fluid flow channel **50** by closing the valves **42A** and **42B** and optionally circulating the reaction mixture within the rotary fluid channel using the circulation pump **34'**. Once the chemical reaction is complete (or after a certain period of time) the valves **42A** and **42B** are opened and the reaction mixture is pumped through the fluid flow channel **18** by the pump **34** into the capillary nozzle **14**, which is connected to a sample injection port **114** of the analytical device **120**. The reaction mixture is then injected into the analytical device **120** and the reaction product(s) are analyzed directly.

In an assay sample preparation, desired compounds, enzymes or cells are immobilized on the surface of sample preparation chamber. Methods for immobilizing

these materials on a solid support containing functional groups such as hydroxides or amines is well known to one of ordinary skill in the art. For example, U.S. Patent Nos. 5,424,186 and 5,959,098, which are incorporated herein by reference in their entirety, describe immobilization of polymers such as oligonucleotides and peptides on solid support. If the inner surface of sample preparation chamber is hydrophobic, it can be functionalized with hydrophilic functional groups. For example, a hydrophobic elastomer surface can be treated with oxygen or water plasma to introduce hydroxy functional groups, which can then be used to immobilize desired materials. After desired materials are immobilized, a mixture of compounds is then introduced into the sample preparation chamber. The mixture of compounds can be exposed to the immobilized material for desired time by circulating the mixture within the sample preparation chamber, or alternatively the mixture of compounds can be exposed to the immobilized material by allowing the mixture to simply flow through the sample preparation chamber (in which case the sample preparation chamber need not be a rotary fluid flow channel). The appropriate target compound then bind to the immobilized material while other compounds are washed away. After removing non-binding compounds, the bound compounds can be released from the immobilized material, e.g., by adding more a strongly binding competitive binding compounds or by denaturing the enzyme to release the bound compounds. The released compounds can then be injected into the analytical device directly and analyzed.

The sample preparation chamber can also be used to degrade proteins into smaller components (e.g., oligonucleotides or amino acids) for analysis. For example, one can integrate a tryptic proteolysis of a peptide on the microfluidic device **100** and inject the resulting sample into the analysis device **120** for analysis. For a representative illustration of a tryptic proteolysis of a peptide see Xue et al., *Rapid Commun. Mass Spectrom.*, **1997**, *11*, 1253, which is incorporated herein by reference in its entirety. In addition, a peptide can be degraded in the sample preparation chamber by immobilizing an enzyme, as described above, which is capable of degrading the peptide and introducing the peptide into the sample preparation chamber. The degraded peptide components can then be effused into a mass spectrometer for analysis. Such peptide sequencing using a mass spectrometer is well known to one of ordinary skill in the art. See for example, Shevchenko et al., *Rapid Commun. Mass Spectrom.*, **1997**, *11*, 1015-1024, which is incorporated herein by reference in its entirety. Briefly, Shevchenko et al. describe a rapid peptide sequencing using a combination of nanoelectrospray quadrupole/time-of

flight mass spectrometer and isotopic labeling of the peptide. By analyzing the mass spectrum pattern of fragments of peptides and comparing the results with known database of peptide mass spectrum patterns, Shevchenko et al. were able to sequence a peptide relatively quickly.

Alternatively, the sample preparation chamber can be a DNA sorter as disclosed by Chou et al., *Proc. Natl. Acad. Sci.*, **1999**, 11-13, or a cell sorter as disclosed in PCT Patent Application Publication No. WO 99/61888, which are incorporated herein by reference in their entirety. Thus, compounds can be sorted based on, e.g., a particular fluorescence wavelength and analyzed by the analytical device.

In addition, by having a portion of the fluid flow channel filled with affinity sieves or similar chromatography material, a mixture of compounds can be separated and each compounds can be analyzed separately.

It should be appreciated that one or more of the above described sample preparation steps can be combined sequentially to provide a variety of sample preparation combinations. For example, sample preparation step can include preparing (i.e., synthesizing) compounds in one sample preparation chamber which is connected to another sample preparation chamber for assaying the compounds, e.g., for enzyme binding. In this manner, a variety of manipulations can be conducted in a single microfluidic device or a combination of microfluidic devices before injecting the analyte into the analytical device for analysis (e.g., identification).

Methods of Fabricating Microfluidic Devices

One exemplary method of fabricating microfluidic devices of the present invention is provided herein, which is similar to methods disclosed in U.S. Patent Application Serial No. 09/605,520, which was previously incorporated by reference. It is to be understood that the present invention is not limited to fabrication by this method. Rather, other suitable methods of fabricating the present microstructures, including modifying the present methods, are also contemplated to be within the scope of the present invention.

Figures 3A and 3B illustrate sequential steps of a preparing a rounded (i.e., circular cross-section) fluid flow channel. Preferably, channels are molded in each layer of the elastomer using soft lithography. A thin layer of photoresist **64** is spin coated on to a wafer **60**. The photoresist is exposed using a high transparency film as a mask (not shown) with desired pattern. The exposed photoresist is then developed to provide a

mold (for clarity only one flow channel mold **64A** is shown). The height of flow channel mold **64A** depends on the thickness of the photoresist. If desired, multiple layers of photoresist can be applied to achieve the desired thickness, using intermediate 'hard bake,' which generally involves heating (e.g., to about 120 °C) to fix channel structures in one part of the device prior to creation of the bottom portion **10** or a second layer **30**. This approach can be used to create regions with different channel depth in different parts of the same device.

Typically, the exposure and development of a photoresist results in a trapezoidal shaped mold. The photoresist is then heated (e.g., at 200 °C for about 30 minutes) to "reflow" the photoresist, thereby producing a rounded flow channel mold **64B**. This "rounding" facilitates sealing of capillaries of different dimensions within the device. A layer of elastomer **20** (i.e., top portion of the first elastic layer) is then spin coated on to the mold, as shown in Figure 3B. After curing, the elastomer is removed from the mold to provide a recess which becomes a part of the flow channel **18**. A complimentary bottom portion elastomer **10** is produced and combined with the top portion **20** to produce a first elastomer layer **25**. A second elastic layer **30** comprising pump **34** and valve **42** systems is then produced as a single layer and bonded together with (i.e., affixed on top of) the first elastic layer **25**.

Upon casting in elastomer, such as GE Silicones RTV615 or Dow Corning Sylgard, a channel is created whose depth is dependent on the thickness of the photoresist upon the wafer. The capillary nozzle **14** is formed by placing a capillary nozzle (e.g., a drawn silica capillary) in the flow channel **18**, so that the capillary nozzle **14** sits within the flow channel **18** and extends beyond the edge of the microfluidic device **100**. Typically, the distance of the capillary nozzle extension beyond the edge of the microfluidic device **100** is from about 50 μm to about 5 mm, preferably from about 100 μm to about 2 mm, and more preferably from about 100 μm to about 1 mm. However, the distance of the extension can be longer depending on a particular application. The capillary **14** is sealed within two portions (Figures 6A and 6B). It can be sealed either directly by baking together the two portions of partially cured elastomers or by incorporation of uncured elastomer (e.g., RTV) during the final bake (i.e., curing) stage.

Fluids are designed to flow in the middle of the two portions (i.e., top portion **20** and bottom portion **10**) of this device. The alignment of the capillary **14** between the two portions and its juxtaposition with the fluid channel **18** can create a

partial occlusion of the capillary if the capillary is perfectly centered between the layers. Better alignments can be achieved by creating an offset in the depths (i.e., height) of two portions of the channels between which the capillary is fitted. For example, if the depth of the photoresist for the lower portion is 5 microns less than the upper portion of the first elastic layer **25**, a capillary with a ten-micron internal diameter can be accommodated without a significant offset.

The portion of fluid flow channel **18** which becomes integrated with the capillary nozzle **14** is configured such that the fluid sample flows directly from the microfluidic device **100** to the analytical device (e.g., mass spectrometer). Additional features patterned in photoresist may be necessary to reduce potential dead volume **16** at the junction between the capillary **14** and the fluid flow channel **18**. Alternative, the amount of dead volume can be reduced by using a tapered capillary as shown in Figure 6C.

This configuration is compatible with commercially available drawn silica capillaries and custom-drawn capillaries. The dimensions of the capillaries that can be accommodated in this configuration include, but are not limited to, capillaries with internal diameters of from about 1 μm to about 100 μm and outer diameters of from about 20 μm to about 360 μm.

In order to create 'pump' and 'valve' features within the microfluidic device **100**, a second elastic layer **30** having 'control line' features (for pumps and valves) is bonded on top of the first elastic layer. This second elastic layer is prepared using a similar process for the above described top or bottom portions of the first elastic layer. Typically, the second elastic layer is then baked (or cured) together with the first elastic layer to create the final device.

The capillary **14** can be sealed within the microfluidic device **100**, by a variety of processes. For example, the capillary **14** can be sealed during baking together of the two portions of the first elastic layer **25**. Alternatively, as shown in Figures 7A-7C, the capillary is 'push-fit' into the device between two portion of the first elastic layer **25**, thereby creating an instant seal. The dimensions of the push-fit envelope are chosen to accommodate the diameter of the capillary **14**. For example, an envelope of about 200 μm width and about 15 μm in height has a perimeter of 430 microns. A capillary with 100 μm outer diameter has a circumference of 314 μm. The seal can be further secured by incorporation of uncured elastomer (e.g., RTV) in the envelope between the two

portions (e.g., areas **22A** and **22B**). As shown in Figure 8, push fitting can also be used to incorporate a capillary that fits into the device in the 'Z' plane. One major advantage of push fitting is that capillaries can be easily interchanged if clogging occurs.

Application of high voltage for electrospray

A high voltage applied to capillary nozzle causes ionization of molecules passing through it at atmospheric pressure and formation of a plasma stream that is accelerated into the analytical device (e.g., mass spectrometer). The system allows for both the sample preparation and sample delivery processes for the ESI-MS to be integrated on the microfluidic device.

Typically application of voltages of from about 500 to about 5 kilovolts (kV) are required to create the electrospray. Two illustrative examples are shown in Figures 9A-9D. One method, as shown in Figures 9A and 9B uses a metallized (e.g., Pd or Gold) or metal coated capillary, which are commercially available. In this method, a high voltage device **110** applies voltage to capillary **14** having a metal coating **12** which creates electrospray **8**. Unfortunately, these types of capillaries have a limited life-span due to evaporation of the metallized layer that carries the charge. Another method uses an external metal sheath capillary as shown in Figures 9C and 9D. In this embodiment, a high voltage device **110** applies voltage to capillary **14** having a metal sheath **12'** to create electrospray **8**. This method has the added benefit that it can be built into a plastic housing for the device and the capillary itself can be used for more than one sample.

Applications:

The integrated microfluidic devices that consist of fluid flow channels, pumps and valves can be used in a variety of applications as discussed above. In addition, such devices can be used as nanoliter-scale fluid delivery devices for reliably delivering highly homogeneous, nanoliter volumes (e.g., from about 1 nL/min to about 200 nL/min) of fluid to the mass spectrometer interface. Such devices can also be used as nanoliter-scale devices which integrate sample purification, separation and processing, as discussed in detail above. This reduces sample preparation cost, avoids sample cross contamination, and enables the application of mass spectrometry to other areas of interest, such as medical diagnostics. Furthermore, such devices can be interfaced with a robotic auto sampler to provide high throughput nanospray device. In one example of this

device, multiple channels are created in the elastomer, each of which is operated by a single set of pumps and individually sampled using a control valve.

Microfluidic devices of the present invention are useful in proteomics such as classic proteomics, e.g., identification and quantitation of unknown proteins identified using 1-D and 2-D gel electrophoresis, and functional proteomics, e.g., analysis of molecular interactions. In addition, microfluidic devices of the present invention are also useful in drug or target molecule discovery. NanoES MS/MS is the most powerful approach currently available, as it allows unambiguous protein and peptide fragment data to be queried against EST and genomic databases. For example, a protein identified as differentially expressed or with variable post-translational modification when two samples or tissues are compared can be identified by comparing the peptide sequences obtained by mass spectrometry against EST and genomic databases.

Moreover, the identified nucleotide sequences, combined with the peptide sequences generated by mass spectrometry, can be used for cloning the protein, in downstream assay development, target validation. And as discussed in detail above, on-chip digestion (i.e., degradation) of proteins with proteases (for example using immobilized trypsin) and on-chip separations can also be achieved by using microfluidic devices of the present invention.

In addition, microfluidic devices of the present invention can be used to assist in drug development by enabling unambiguous identification of metabolites in serum, urine, etc. Furthermore, microfluidic devices of the present invention can be used in ADME/PK (absorption-distribution-metabolism-excretion/pharmacokinetic) studies. Additionally, high throughput screening can be conducted directly by using the MS to provide assay readout.

Other uses for microfluidic devices of the present invention include, but are not limited to, applications in genomics, e.g. high throughput genotyping, applications in analytical chemistry, on chip separations, on-chip combinatorial chemistry, and analysis of proteins in clinical diagnostics.

In particular, the sample preparation chamber can be used for conducting a chemical reaction; conducting an assay; degrading a peptide or protein; conducting a chemical analysis; extraction of analytes from solvents (aqueous/non-aqueous); extraction of analytes from bodily fluids; concentration of sample analytes; affinity purification of an analyte; digesting a nucleic acid, carbohydrate, lipid or other molecule or mixture of molecules; separation; and cell growth (mammalian, bacterial or parasite).

In combinatorial synthesis, microfluidic devices of the present invention can us a monomer (i.e., starting material) that is selected from the group consisting of nucleotides, amino acid peptides, carbohydrates, lipids, and other precursors for combinatorial synthesis.

The sample preparation step can also comprise binding a target molecule to an array of oligonucleotides, peptides, proteins, oligosaccharides, and small molecules (e.g., drugs).

Preferred Layer and Channel Dimensions:

Microfabricated refers to the size of features of an elastomeric structure fabricated in accordance with an embodiment of the present invention. In general, variation in at least one dimension of microfabricated structures is controlled to the micron level, with at least one dimension being microscopic (i.e. below 1000 μm). Microfabrication typically involves semiconductor or MEMS fabrication techniques such as photolithography and spin coating that are designed for to produce feature dimensions on the microscopic level, with at least some of the dimension of the microfabricated structure requiring a microscope to reasonably resolve/image the structure.

For integration with an electrospray capillary for use in a mass spectrometer, preferred width-to-depth ratios of a rectangular cross-section fluid flow channel **18** is from about 0.1:1 to about 100:1, more preferably from about 1:1 to about 50:1, still more preferably from about 2:1 to about 20:1, and most preferably from about 3:1 to about 15:1. For a circular cross-section fluid flow channel **18**, preferred diameter is from about 1 μm to about 500 μm, more preferably from about 1 μm to about 200 μm, and most preferably from about 3 μm to about 200 μm.

The flow channels are not limited to these specific dimension ranges and examples given above, and can vary depending on a particular sample delivery means employed. For example, wider flow channels having a diameter in the order of about 1000 μm may be useful in other analytical device interface, such as HPLC or UV spectrometer.

The thickness of the first elastomeric layer **25** also depends on a particular application. For use in electrospray-MS with nL/min sample deliver rate, the first elastomeric layer **25** has thickness of from about 40 μm to about 10 mm, preferably from about 40 μm to about 5mm, and more preferably from about 40 μm to about 3 mm.

Accordingly, the layer of elastomer separating the flow channel **18** and the pressure channel (e.g., pumps and valves) has a typical thickness of from about 0.01 μm to about 1000 μm, preferably from about 0.05 μm to about 500 μm, more preferably from about 0.2 μm to about 250 μm, still more preferably from about 1 μm to about 100 μm, yet still more preferably from about 2 μm to about 50 μm, and most preferably from about 5 μm to about 40 μm.

The pressure channels that make up the pump and valve systems typically have rectangular cross-section for ease of fabrication. However, the cross-section is not limited to such shape. Preferably the width of pressure channels is from about 0.01 μm to about 1000 μm, preferably from about 0.05 μm to about 1000 μm, more preferably from about 0.2 μm to about 500 μm, still more preferably from about 1 μm to about 250 μm, and most preferably from about 10 μm to about 200 μm. The thickness of the second elastomeric layer **30** is from about 50 μm to several centimeters, preferably from about 0.1 μm to about 10 cm, more preferably from about 1 μm to about 5 cm, still more preferably from about 10 μm to about 2 cm, and most preferably from about 100 μm to about 10 mm.

Multilayer Soft Lithography Construction Techniques and Materials:

Preferably, elastomeric portions **10** and **20** (and elastomeric layer **25** and **30**) are bonded together chemically, using chemistry that is intrinsic to the polymers comprising the patterned elastomer layers. Most preferably, the bonding comprises two component "addition cure" bonding.

In a preferred aspect, the layers (or portions) of elastomer are bound together in a heterogenous bonding in which the layers have a different chemistry. Alternatively, a homogenous bonding can be used in which all layers would be of the same chemistry. Thirdly, the respective elastomer layers can optionally be glued together by an adhesive instead. In a fourth aspect, the elastomeric layers can be thermoset elastomers bonded together by heating.

In one aspect of homogeneous bonding, the elastomeric layers are composed of the same elastomer material, with the same chemical entity in one layer reacting with the same chemical entity in the other layer to bond the layers together. In one embodiment, bonding between polymer chains of like elastomer layers can result

from activation of a crosslinking agent due to light, heat, or chemical reaction with a separate chemical species.

Alternatively in a heterogeneous aspect, the elastomeric layers are composed of different elastomeric materials, with a first chemical entity in one layer reacting with a second chemical entity in another layer. In one exemplary heterogenous aspect, the bonding process used to bind respective elastomeric layers together can comprise bonding together two layers of RTV 615 silicone. RTV 615 silicone is a two-part addition-cure silicone rubber. Part A contains vinyl groups and catalyst; part B contains silicon hydride (Si-H) groups. The conventional ratio for RTV 615 is 10A:1B. For bonding, one layer can be made with 30A:1B (i.e. excess vinyl groups) and the other with 3A:1B (i.e. excess Si-H groups). Each layer is cured separately. When the two layers are brought into contact and heated at elevated temperature, they bond irreversibly forming a monolithic elastomeric substrate.

In an exemplary aspect of the present invention, elastomeric structures are formed utilizing Sylgard 182, 184 or 186, or aliphatic urethane diacrylates such as (but not limited to) Ebecryl 270 or Irr 245 from UCB Chemical.

Alternatively, other bonding methods can be used, including activating the elastomer surface, for example by plasma exposure, so that the elastomer layers/substrate bond when placed in contact. For example, one approach to bonding together elastomer layers composed of the same material is set forth by Duffy et al, "Rapid Prototyping of Microfluidic Systems in Poly(dimethylsiloxane)", *Analytical Chemistry*, **1998**, *70*, 4974-4984, which is incorporated herein by reference in its entirety. This paper discusses that exposing polydimethylsiloxane (PDMS) layers to oxygen plasma causes oxidation of the surface, with irreversible bonding occurring when the two oxidized layers are placed into contact.

Yet another approach to bonding together successive layers of elastomer is to utilize the adhesive properties of uncured elastomer. Specifically, a thin layer of uncured elastomer such as RTV 615 is applied on top of a first cured elastomeric layer. Next, a second cured elastomeric layer is placed on top of the uncured elastomeric layer. The thin middle layer of uncured elastomer is then cured to produce a monolithic elastomeric structure. Alternatively, uncured elastomer can be applied to the bottom of a first cured elastomer layer, with the first cured elastomer layer placed on top of a second cured elastomer layer. Curing the middle thin elastomer layer again results in formation of a monolithic elastomeric structure.

Suitable Elastomeric Materials:

Allcock et al, *Contemporary Polymer Chemistry*, 2nd Ed. describes elastomers in general as polymers existing at a temperature between their glass transition temperature and liquefaction temperature. Elastomeric materials exhibit elastic properties because the polymer chains readily undergo torsional motion to permit uncoiling of the backbone chains in response to a force, with the backbone chains recoiling to assume the prior shape in the absence of the force. In general, elastomers deform when force is applied, but then return to their original shape when the force is removed. The elasticity exhibited by elastomeric materials may be characterized by a Young's modulus. Elastomeric materials having a Young's modulus of between about 1 Pa – 1 TPa, more preferably between about 10 Pa – 100 GPa, still more preferably between about 20 Pa – 1 GPa, yet more preferably between about 50 Pa – 10 MPa, and most preferably between about 100 Pa – 1 MPa are useful in accordance with the present invention. It should be appreciated, however, elastomeric materials having a Young's modulus outside of these ranges can also be utilized depending upon the needs of a particular application.

Microfluidic devices of the present invention can be fabricated from a wide variety of elastomers. In an exemplary aspect, elastomeric layers **25** and **35** are preferably fabricated from silicone rubber. However, other suitable elastomers may also be used.

In an exemplary aspect of the present invention, microfluidic devices are fabricated from an elastomeric polymer such as GE RTV 615 (formulation), a vinyl-silane crosslinked (type) silicone elastomer (family). However, microfluidic devices of the present invention are not limited to this one formulation, type or even this family of polymer; rather, nearly any elastomeric polymer is suitable. In the case of multilayer soft lithography, preferably layers of elastomer are cured separately and then bonded together. This scheme requires that cured layers possess sufficient reactivity to bond together. Either the layers can be of the same type, and are capable of bonding to themselves, or they can be of two different types, and are capable of bonding to each other. Other possibilities include the use an adhesive between layers and the use of thermoset elastomers.

Given the tremendous diversity of polymer chemistries, precursors, synthetic methods, reaction conditions, and potential additives, there are a huge number of possible elastomer systems that could be used to make microfluidic devices of the

present invention. Variations in the materials used depends on the need for particular material properties, i.e. solvent resistance, stiffness, gas permeability, or temperature stability.

There are a variety of types of elastomeric polymers. A brief description of the most common classes of elastomers is presented here, with the intent of showing that even with relatively "standard" polymers, many possibilities for bonding exist. Common elastomeric polymers include polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicones.

Polyisoprene, polybutadiene, polychloroprene:

Polyisoprene, polybutadiene, and polychloroprene are all polymerized from diene monomers, and therefore have one double bond per monomer when polymerized. This double bond allows the polymers to be converted to elastomers by vulcanization (essentially, sulfur is used to form crosslinks between the double bonds by heating). This would easily allow homogeneous multilayer soft lithography by incomplete vulcanization of the layers to be bonded; photoresist encapsulation would be possible by a similar mechanism.

Polyisobutylene:

Pure polyisobutylene has no double bonds, but is crosslinked to use as an elastomer by including a small amount (~1%) of isoprene in the polymerization. The isoprene monomers give pendant double bonds on the polyisobutylene backbone, which may then be vulcanized as above.

Poly(styrene-butadiene-styrene):

Poly(styrene-butadiene-styrene) is produced by living anionic polymerization (that is, there is no natural chain-terminating step in the reaction), so "live" polymer ends can exist in the cured polymer. This makes it a natural candidate for the present photoresist encapsulation system (where there will be plenty of unreacted monomer in the liquid layer poured on top of the cured layer). Incomplete curing would allow homogeneous multilayer soft lithography (A to A bonding). The chemistry also facilitates making one layer with extra butadiene ("A") and coupling agent and the other layer ("B") with a butadiene deficit (for *hetero*geneous multilayer soft lithography). SBS is a "thermoset elastomer",

meaning that above a certain temperature it melts and becomes plastic (as opposed to elastic); reducing the temperature yields the elastomer again. Thus, layers can be bonded together by heating.

Polyurethanes:

Polyurethanes are produced from di-isocyanates (A-A) and di-alcohols or di-amines (B-B); since there are a large variety of di-isocyanates and di-alcohols/amines, the number of different types of polyurethanes is huge. The A vs. B nature of the polymers, however, would make them useful for heterogeneous multilayer soft lithography just as RTV 615 is: by using excess A-A in one layer and excess B-B in the other layer.

Silicones:

Silicone polymers probably have the greatest structural variety, and almost certainly have the greatest number of commercially available formulations. The vinyl-to-(Si-H) crosslinking of RTV 615 (which allows both heterogeneous multilayer soft lithography and photoresist encapsulation) has already been discussed, but this is only one of several crosslinking methods used in silicone polymer chemistry.

Cross Linking Agents:

In addition to the use of the simple "pure" polymers discussed above, crosslinking agents can also be added. Some agents (like the monomers bearing pendant double bonds for vulcanization) are suitable for allowing homogeneous (A to A) multilayer soft lithography or photoresist encapsulation; in such an approach the same agent is incorporated into both elastomer layers. Complementary agents (i.e. one monomer bearing a pendant double bond, and another bearing a pendant Si-H group) are suitable for heterogeneous (A to B) multilayer soft lithography. In this approach complementary agents are added to adjacent layers.

Other Materials:

In addition, polymers incorporating materials such as chlorosilanes or methyl-, ethyl-, and phenylsilanes, and polydimethylsiloxane (PDMS) such as Dow

Chemical Corp. Sylgard 182, 184 or 186, or aliphatic urethane diacrylates such as (but not limited to) Ebecryl 270 or Irr 245 from UCB Chemical can also be used.

The following is a non-exclusive list of elastomeric materials which can be utilized in connection with the present invention: polyisoprene, polybutadiene, polychloroprene, polyisobutylene, poly(styrene-butadiene-styrene), the polyurethanes, and silicone polymers; or poly(bis(fluoroalkoxy)phosphazene) (PNF, Eypel-F), poly(carborane-siloxanes) (Dexsil), poly(acrylonitrile-butadiene) (nitrile rubber), poly(1-butene), poly(chlorotrifluoroethylene-vinylidene fluoride) copolymers (Kel-F), poly(ethyl vinyl ether), poly(vinylidene fluoride), poly(vinylidene fluoride – hexafluoropropylene) copolymer (Viton), elastomeric compositions of polyvinylchloride (PVC), polysulfone, polycarbonate, polymethylmethacrylate (PMMA), and polytertrafluoroethylene (Teflon).

Doping and Dilution:

Elastomers can also be "doped" with uncrosslinkable polymer chains of the same class. For instance RTV 615 may be diluted with GE SF96-50 Silicone Fluid. This serves to reduce the viscosity of the uncured elastomer and reduces the Young's modulus of the cured elastomer. Essentially, the crosslink-capable polymer chains are spread further apart by the addition of "inert" polymer chains, so this is called "dilution". RTV 615 cures at up to 90% dilution, with a dramatic reduction in Young's modulus.

Pre-Treatment and Surface Coating

Once the elastomeric material has been molded or etched into the appropriate shape, it can be pre-treated in order to facilitate operation in connection with a particular application. For example, in sorting biological entities such as cells or DNA the hydrophobic nature of the biological entity can cause it to adhere to the hydrophobic elastomer of the walls of the channel. Therefore, it is useful to pre-treat the elastomeric structure order to impart a hydrophilic character to the channel walls. In an embodiment of the present invention utilizing the General Electric RTV 615 elastomer, this can be accomplished by boiling the shaped elastomer in acid (e.g. 0.01% HCl in water, pH 2.7, at 60 °C for 40 min).

Other types of pre-treatment of elastomer material are also contemplated by the present application. For example, certain portions of elastomer can be pre-treated to create anchors (i.e., immobilization site) for surface chemistry reactions (for example in the formation of peptide chains), or binding sites for antibodies.

Methods of Operating Microfluidic Devices of the Present Invention

Methods for pumping fluids and opening or closing valve systems are disclosed in the above mentioned U.S. Patent Application Serial No. 09/605,520, which was previously incorporated by reference in its entirety.

The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, *e.g.*, as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety.